REMARKS

I. Claim Amendments

Claims 35, 39, 40, 42-50, 52-54 and 57-73 are currently pending. Claims 1-34, 36-38, 41, 51, 55 and 56 have been canceled previously. With this response, claims 42 and 43 have been canceled, claims 35, 61, 70-72 have been amended and claims 74-78 have been added to define more clearly what applicant considers to be his invention. Each of these amendments is supported by the specification as originally filed and none adds new matter. The amendments are addressed below in the context of addressing the Examiner's outstanding rejections.

A. 35 U.S.C. § 112 Claim Rejections – Written Description

Claims 35, 39, 40, 42-50, 52-54 and 57-69 and 71-73 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of adequate written support in the application as originally filed. The Examiner acknowledges that the written description requirement has been met for a "lower eukaryotic host cell that does not display *alpha-1,6 mannosyltransferase activity* with respect to the N-glycan on a glycoprotein" (Action, page 4). But the Examiner points out that the rejected claims are not limited to the alpha-1,6 mannosyltransferase, as they recite a "lower eukaryotic host cell that does not display *a 1,6 mannosyltransferase activity* with respect to the N-glycan on a glycoprotein". And, according to the Examiner, "[t]he specification does not disclose a lower eukaryotic host cell lacking any activity of other 1,6 mannosyltransferase with respect to N-glycan of the glycoprotein" (*Id.*). Applicant has amended claim 35 to limit the recited 1,6-mannosyltransferase activity to an "alpha-1,6 mannosyltransferase" activity, thus obviating this rejection. Applicant notes that pending independent claim 71 (and hence dependent claim 72)

already recites the "alpha" limitation and hence has not been further amended. New claims 74-78 also recite that the lower eukaryotic host cell "does not display an alpha-1,6 mannosyltransferase activity with respect to the N-glycan on a glycoprotein." Accordingly, applicant requests that the Examiner withdraw the 112 rejections based on this term.

Claims 35, 39, 40, 42-50, 52-54 and 57-69 and 71-73 also stand rejected under 35 U.S.C. § 112, first paragraph, for lack of adequate written support in the application with respect to "a mannosidase enzyme for the production of a $Man_5GlcNAc_2$ carbohydrate structure." The Examiner states that "[a]lthough the specification provides a number of alpha 1,2 mannosidase from different species, it still does not satisfy the written description requirement for any hybrid mannosidase enzymes as claimed." (Action, page 5). Accordingly, applicant has amended claims 35 and 71 to specify that the mannosidase enzyme introduced into the host cell has alpha 1,2 mannosidase activity.

Specifically, each of amended claims 35 and 71 recites: "the enzyme comprising: (a) a catalytic domain having alpha-1,2 mannosidase activity." New claims 74-78 also require that the host cell display alpha-1,2, mannosidase activity. In addition, for clarity, applicant has deleted the term "hybrid" from the claims, as the term is redundant with the recitation that the claimed enzyme comprise (a) a "catalytic domain" and (b) a "cellular targeting signal peptide not normally associated with the catalytic domain."

Based on the above amendments, applicant respectfully requests that the Examiner withdraw the outstanding claim rejections based on lack of adequate written description.

B. 35 U.S.C. § 112 Claim Rejections – Enablement

Claims 35, 39, 40, 42-50, 52-54 and 57-73 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. In particular, the Examiner contends that the specification "does not reasonably provide enablement for a method for producing *any* type of humanized protein with *any* type of complex glycoprotein structure (even when the intermediate $Man_5GlcNAc_2$ structure is produced.) (Action, page 6; emphasis added.) The Examiner also contends that the specification does not enable humanized protein production "without introducing [an] exogenous construct encoding such protein." *Id.* The Examiner affirms, however, that the original specification *does* enable methods for producing glycoproteins in lower eukaryotic host cells that have been transfected or co-transfected with nucleic acid constructs that encode the protein of interest to be glycosylated, and one or more of: alpha-1,2 mannosidases I and II, N-acetylglucosaminyl transferases I (GnTI) and II (GnTII), and a UDP-acetylglucosamine transporter. As detailed below, although applicant traverses these enablement rejections, applicant's claim amendments nonetheless obviate the Examiner's rejections.

Claims 35, 54, 59, 70 and 71 have been amended to delete the term "humanized" and replace it with "recombinant." As amended, claims 35, 70 and 71 thus recite "[a] method for producing a recombinant glycoprotein" and dependent claims 54 and 59 refer to "production of the glycoprotein." New claims 74-76 also contain this phrase. Support for this amendment is found, e.g., at page 11, lines 28-30 (see also, e.g., page 8, line 26; page 9, lines 7 and 11; and page 12, line 6) of the original specification.

Deletion of the term "humanized" overcomes the Examiner's rejection based on lack of enablement to produce "any type of humanized protein with *any* type of complex glycoprotein structure (even when the intermediate $Man_5GlcNAc_2$ structure is produced.)" (Action, page 6.)

Addition of the term "recombinant" overcomes the Examiner's rejection based on lack of enablement to produce "*any* type of humanized protein," as the amended claims are now limited to recombinantly expressed proteins. Addition of the term "recombinant" also overcomes the Examiner's rejection based on her assertion (which is traversed[1]) that the specification fails to enable a method of producing a humanized protein in a lower eukaryotic host cell without introducing an exogenous construct encoding such a protein. In addition, claim 35 has been amended to recite that it is "upon expression of the recombinant glycoprotein in the host cell" that the desired modified N-glycan structure is produced. The claims as amended herein no longer encompass a method for producing *any* type of humanized protein with *any* type of complex glycoprotein structure. Applicant thus respectfully requests that the Examiner withdraw her rejections on this basis.

The Examiner has also rejected claims 71-73 for lack of enablement because "the specification does not teach how to convert long mannose chain N-glycan structure to humanized glycoprotein without the trimming by mannosidase to first produce the intermediate $Man_5GlcNAc_2$ structure." (Action, page 6.) Claim 71 has thus been amended to recite that the method comprises

[1] The claimed methods may be used to produce recombinant glycoproteins in lower eukaryotic host cells that have been *previously* engineered to express a recombinant protein. And, in fact, the invention is not limited to producing a protein-of-interest from an exogenous DNA introduced into the host cell. Host cell endogenous proteins that are destined to be secreted will also enter the host's engineered secretory pathway and become glycosylated with human-like N-glycan structures.

"the step or steps of introducing into the host cell at least two enzymes," one having alpha-1,2 mannosidase activity and the other having GlcNAc transferase I activity. Support for this amendment is found throughout the original specification; see, e.g., page 14, lines 11-23, and page 16, lines 6-21; page 20, line 8 – page 25, line 20; and Example 2 at page 33. Amended claims 71-73 (and new claims 74-76, see below) each recite a method by which the host cell is capable of making the N-glycan substrate for the enzyme that is being introduced into the host cell. Thus, the Examiner's rejections of claims 71-73 have been obviated.

New claims 74-77 each recite a method in which a lower eukaryotic host cell which displays a particular set of glycosylation enzyme activities receives an additional enzyme selected for optimal activity in the ER or Golgi apparatus of the particular host cell being used, the additional enzyme comprising (a) a catalytic domain selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in the subcellular location where the domain is targeted; and "(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to said ER or Golgi apparatus." As discussed above, these claims each recite a method by which the host cell is capable of making the N-glycan substrate for the enzyme that is being introduced into the host cell. Original support for new claims 74-77 is found throughout the original specification; see, e.g., page 14, lines 14-21; page 16, lines 6-21 (and see page 2, lines 15-26 for "complex N-glycans"); page 25, line 15 to page 26, line 6; page 27, lines 5-18 and Examples 2-7 at pages 33-38; see also original claims 12-16.

Post-filing date evidence of enablement

Finally, applicant acknowledges with appreciation the Examiner's recognition that "the claimed method is enabled to the extend (*sic*) of what is demonstrated in Choi et al. and Hamilton et al." (Action, page 8.) Applicant respectfully traverses, however, the Examiner's statement that "the specification itself does not provide sufficient enablement for the claimed invention" and any suggestion by the Examiner that it is only the post-filing date evidence that provides enablement. (*Id.*) Rather, Choi et al. and Hamilton et al. references provide post-filing date evidence demonstrating that the original application *as filed* enabled the claimed invention, even though not all of the working examples had been provided at the filing date. There is no requirement that an application provide working examples in order to enable a claimed invention (*see* MPEP § 2164.02).

New claims 74-77 are also supported by the application as filed, and encompass methods of the invention demonstrated by Choi et al. and Hamilton et al. to produce recombinant glycoproteins having N-glycans with $GlcNAcMan_5GlcNAc_2$ (claim 74), $GlcNAcMan_3GlcNAc_2$ (claim 75) and $GlcNAc_2Man_3GlcNAc_2$ (claim 76) glycoforms. New claim 77, which depends from new claims 74-76, further requires that one or more of the glycosylation enzymes responsible for producing such N-glycans (alpha-1,2 mannosidase, GlcNAc transferase I, GlcNAc transferase II and mannosidase II) be expressed from exogenous DNA introduced into the lower eukaryotic host cell.

Based on the above, applicant respectfully requests that the Examiner withdraw the outstanding enablement rejections.

C. 35 U.S.C. § 112 Claim Rejections – Indefiniteness

Claims 58 and 61 remain rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the term "derived." Applicant has deleted this term from the claims, thus obviating the rejections.

Claims 35, 39, 40, 42-50, 52-54, and 57-70 stand rejected under 35 U.S.C. § 112, second paragraph, for being unclear and incomplete for failing to bridge the gap between steps that produce a $Man_5GlcNAc_2$ glycoform and steps that produce a desired "humanized" glycoprotein.

Applicant traverses the Examiner's characterization that a "gap" exists between the $Man_5GlcNAc_2$ "intermediate" and "humanized" glycoforms, which is contrary to the teaching of the application as a whole (the application teaches that the $Man_5GlcNAc_2$ "intermediate" is "humanized" compared to N-glycans made in a wild-type lower eukaryotic host cell.) Nonetheless, as amended, those claims no longer characterize the glycoprotein as being "humanized." Deletion of the term "humanized" from claims 35, 54, 59 and 70 (and deletion of "human-like" from claim 71) thus obviates the Examiner's rejection with respect to the use of this term. As amended, the claims clearly recite that glycoproteins comprising desired glycoform structures have been produced in host cells expressing glycoproteins comprising a $Man_5GlcNAc_2$ N-glycan structure. In certain embodiments, (i.e., if the host cell displays additional glycosylation enzymatic activities as taught in the application), the $Man_5GlcNAc_2$ N-glycan structure may be further processed within the host cell (e.g., to $GlcNAcMan_5GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$ and/or $GlcNAc_2Man_3GlcNAc_2$.) While these claim amendments also obviate the Examiner's rejection of claims 42 and 43 for being unclear and redundant, claims 42 and 43 have been canceled.

D. Amendments For Further Clarity

Applicant has amended independent claims 35, 70 and 71 to recite more clearly what he considers to be the invention. In particular, as amended, claims 35, 70 and 71 now specify that the introduced glycosylation enzyme comprises a specified catalytic domain and a "cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to the ER or Golgi apparatus of the host cell." Original support for these amendments may be found throughout the specification, e.g., at page 12, lines 13-19; page 14, lines 3-6 and 11-13; page 21, lines 3-16; page 28, lines 1 – page 30, line 3; and page 31, lines 7-12; original claim 1.

Claim 48 has been amended to specify that the host cell does not express an "enzyme activity with respect to the N-glycan on a glycoprotein," for clarity and consistency with the language of claim 35, from which it depends.

Finally, applicant has added claim 78 to encompass engineered host cells of the invention made by the methods of claims 35-77. Although the Examiner restricted out claims to lower eukaryotic host cells in the October 2, 2002 Restriction Requirement, claim 78 is a product-by-process claim, and covers products that cannot be made by any other method. The products of the claimed methods are themselves novel, as their glycosylation is a function of the host cell in which they are produced. As such, applicant believes he is entitled to present this claim in the same application as that in which the method claims are presented.

II. Conclusion

Entry of this Amendment and allowance of the claims as submitted herewith is respectfully requested.

Respectfully submitted,

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